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ABSTRACTS OF PAPERS

Thirteenth Annual Albert L. Tester Memorial Symposium 14–15 April 1988[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester who, at the time of his death in 1974, was Senior Professor of Zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields in marine biology. Papers reporting original research on any aspect of biology are solicited from students at the university and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best papers by graduate students. Papers are judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Eric Davidson of the University of California at Los Angeles participated in the symposium.

Osmotic Effect of Formamide on Sodium Channel Gating Currents in Crayfish Giant Axon[2]

D. A. Alicata[3]

Schauf and Chuman (1986, pp. 3–23 *in* Ion channels in neural nembranes, A. R. Liss, Inc., N.Y.) have reported 20% formamide to be an effective pharmacological tool for removing the fast component of the sodium channel gating current in *Myxicola* giant axons. Changing the internal perfusate from the standard 430 milliosmole solution to 5% and 10% formamide (1300 milliosmole and 2600 milliosmole, respectively) reduces the fast component as noted by Schauf and Chuman. However, when high internal osmolarity is controlled with 1300 and 2000 milliosmole sucrose, sucrose is equally effective in reducing the fast component of the gating current.

Stimers et al. (1987, J. Gen. Physiol. 89: 521) have noted uncovering of the fast component in squid axons associated with external hyperosmotic environments. These changes observed in the gating current were proposed to be associated with a decrease in series resistance (Rs). Internal hyperosmotic media might therefore be expected to increase Rs. We note that an imposed increase in Rs slows clamp speed in our system, while reducing clamp speed reversibly obscures the fast

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[3] University of Hawaii, Department of Physiology and Bekesy Laboratory of Neurobiology.

component of gating current. Thus we find no evidence to indicate a direct pharmacological effect of formamide on gating currents in the crayfish giant axon.

Determining the Lethal Dose (LD_{50}) of *Vibrio* and *Pseudomonas* Bacteria for Marine Shrimp[4]

CARL ARUME[5]

The primary purpose of this study was to determine the lethal dose at which 50% mortality occurs (LD_{50}) of eight *Vibrio* isolates. These isolates were obtained from wild harvested marine shrimp and from marine shrimp aquaculture facilities.

The *Vibrio* isolates were tested against *Penaeus stylirostrus*. A standard curve of dilution versus absorbance was made for each isolate. Each isolate was again grown up in 100 ml of nutrient broth, centrifuged, and resuspended in the broth to get 10.0 ml. The absorbance of the 10^{-2} dilution was measured at 600 nm and compared to the standard curve to determine the titer of bacteria in the new solution. Dilutions were adjusted so that a 0.1-ml injection would give 1×10^x bacteria per gram of shrimp (x = the log 10 of the dilution). Ten shrimp (five per tank) were injected with appropriate concentrations of bacteria to give 10^6, 10^5, 10^4, 10^3, 10^2, and 10^1 cells per ml per gram weight of shrimp. A saline solution was used as a control, and the incubation period was 72 hr.

The LD_{50} of the *Vibrio* isolates ranged from 4.0×10^2 to 3.3×10^4. Two *Pseudomonas* species had similar LD_{50}'s. *Escherichia coli* had an LD_{50} of 1.0×10^6. Heat-killed *Vibrio* did not produce mortalities. The injected bacteria were recovered from the blood of moribund animals.

LD_{50}'s of the *Vibrio* isolates were determined. Live cells are needed to produce pathological effect. Considerably fewer *Vibrio* bacteria were required to cause pathogenicity compared to *E. coli*. However, certain pseudomonad species show pathogenicity similar to that of the *Vibrio* isolates. Different species of *Vibrio* and different strains of the same species of *V. alginolyticus* have different levels of pathogenicity on *P. stylirostrus*.

Rapid Food-limited Larval Growth: Implications for the Planktonic Period[6]

JANICE L. BELL[7]

The larval period has long been considered to play an important role in the distribution and abundance of benthic marine invertebrate populations. Dispersal potential, recruitment success, speciation, and biogeography are all influenced by length of the larval period. Because it is difficult to study most marine invertebrate larvae in the field, little is known about their longevity and survival in the plankton. Thus, rates of growth and timing of metamorphosis have been determined by laboratory culture. However, laboratory growth experiments contain higher phytoplankton concentrations, lower phyto-

[4] These studies were funded by the University of Arizona Environmental Research Laboratory and Marine Culture Enterprise. Sponsor: Dr. Roger Fujioka.

[5] University of Hawaii, Department of Microbiology.

[6] This research was supported by the Charles and Margaret Edmondson Research Fund and the International Women's Fishing Association Scholarship. Sponsor: Dr. Michael Hadfield.

[7] University of Hawaii, Department of Zoology.

plankton diversity, and higher larval densities than those found in natural seawater. The question of how fast larvae grow in the ocean and what limits their growth and larval life span was addressed by altering three culture variables: food quality, food concentration, and larval density. Two prosobranch gastropods, *Crucibulum spinosum* and *Crepidula aculeata*, were chosen to compare growth rates and survival in typical culture conditions and those approximating natural seawater. Typical culture conditions consisted of one larva per 3 ml in filtered seawater with a three-species diet of cultured phytoplankton at a density of 1×10^4 cells per ml. Under those conditions metamorphosis occurred 24 days after hatching. Laboratory-line seawater was inadequate for growth and survival of larvae even when provided constantly in a drip-through system. Particle counts showed laboratory-line seawater to contain one-half the number of particles in ocean seawater collected in buckets from a seawall. In the first experiment, this ocean seawater was compared to the cultured phytoplankton diet at various larval concentrations. Larvae metamorphosed in 10 days in ocean seawater at a density of one per 50 ml but grew slowly at a density of one per 10 ml, never reaching metamorphic size by day 25. Larvae fed phytoplankton in filtered seawater grew at the same rate at densities of one per 10 ml as they did at one per 3 ml, metamorphosing at day 24. In a second experiment, larval concentration was held constant at one per 50 ml. Those fed enhanced ocean seawater (phytoplankton added) grew at the same rate as those fed phytoplankton in filtered seawater, but they metamorphosed earlier (6 versus 10 days). Those fed only ocean seawater did not grow as rapidly as those in experiment 1 and failed to reach metamorphic size by the termination of the experiment. Thus, larvae are not starving in the ocean and can grow faster in the ocean than laboratory culture techniques have suggested. However, larval growth is food-limited and the potential exists to utilize patchy food sources for more rapid growth.

Prolactin Cell Function in Tilapia, *Oreochromis mossambicus*[8]

RUSSELL J. BORSKI[9]

Prolactin (PRL), a pituitary hormone, is thought to play important roles in the growth, reproduction, and freshwater osmoregulation of euryhaline teleost fish. In tilapia, there is evidence that PRL secretion is regulated by a variety of factors that include somatostatin (SRIF), osmotic pressure, and thyrotropin-releasing hormone (TRH). Small physiological reductions in medium osmotic pressure increase PRL release from the tilapia pituitary in vitro. TRH stimulates PRL release in vitro from the tilapia pituitary preincubated with estradiol. Alterations in PRL release by reduced osmotic pressure and SRIF appear to be mediated in part by Ca^{++}. However, the role of Ca^{++} in TRH-stimulated PRL release has not been investigated.

To determine whether TRH-stimulated PRL release is dependent on extracellular Ca^{++}, PRL tissues were pretreated for 48 hr in static culture with media containing 50 nM estradiol and then exposed to complete and Ca^{++}-depleted media containing 50 nM TRH for 20 hr. The response to TRH in Ca^{++}-depleted media was significantly reduced, suggesting that extracellular Ca^{++} may be important in coupling TRH stimulus to PRL release.

In tilapia, intracellular Ca^{++} may also play a significant role in mediating TRH-stimulated PRL release. To measure changes in intracellular free Ca^{++} by microspectrofluorometry, individual PRL cells must first be isolated. It was necessary, therefore, to develop a tech-

[8] This research was supported by NSF grant DCB-8615659. Sponsor: Dr. Gordon Grau.

[9] University of Hawaii, Department of Zoology and the Hawaii Institute of Marine Biology.

nique for culturing single PRL cells in vitro and to determine whether these cells respond to established regulators of prolactin release in a manner that is similar to that of intact tissues.

A method of dispersing pituitary tissues has been developed that includes a trypsin digestion of the anterior portion of the tilapia pituitary (rostral pars distalis), followed by an 18-hr preincubation period in hyperosmotic medium, and a final 18-hr incubation in defined media containing hormone treatments. Whole tissues were exposed to the same media and incubation periods as those of dispersed cells. The dispersed cell culture method produced a high yield of individual, viable prolactin cells. Responses of whole tissues and dispersed cells to SRIF and osmotic pressure were virtually identical. Studies are currently underway to determine whether intact tissues and dispersed cells respond similarly to TRH.

Cardiovascular and Respiratory Responses to Acute Hypoxia in Restrained and Free-swimming Tunas[10]

PETER G. BUSHNELL[11]

Tunas have evolved physiological mechanisms that allow them to extract and move large amounts of oxygen from the water to their tissues. The ability of tuna to consume oxygen at rates that approach mammalian standards (2000 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$) enables them to reach burst swimming speeds exceeding 20 body-lengths/sec (BL/s) (approximately 48 km/hr) as well as to maintain speeds of 6 BL/s during transoceanic migrations. Because the open ocean is heterogeneous in terms of oxygen content, it is not unusual for tuna to encounter areas of low ambient oxygen. The aim of this study was to determine how these highly active, oxygen-dependent animals respond physiologically to low ambient oxygen (hypoxia).

Cardiac output (Q), heart rate (HR), ventilation volume (V_g), and gape were measured in restrained yellowfin (*Thunnus albacares*) and skipjack (*Katsuwonus pelamis*) tuna spinally blocked with Lydocaine and subjected to brief periods (approx. 2 min) of reduced oxygen partial pressures (PO_2), varying from 140 to 50 mm Hg (air-saturated water = 154 mm Hg). Both species of fish responded to hypoxia by increasing gape and V_g and decreasing HR and Q, all of which were graded to the severity of the hypoxia. Cardiovascular and respiratory responses were variable in magnitude, but immediate and sensitive to declines in inflow PO_2 of as little as 10 mm Hg.

To validate some of the physiological data recorded in restrained fish, heart rate (measured via an FM transmitter) and swimming speed were measured in free-swimming yellowfin and skipjack tuna while oxygen tension was reduced to approximately 90 mm Hg over a 30-min test period. Heart rate of swimming tuna in air-saturated seawater was 25% lower than that measured in spinally blocked fish. In general, a reduction in ambient PO_2 of 5% was sufficient to produce a significant decrease in HR and simultaneous increase in swimming speed.

Skipjack and yellowfin tuna are sensitive to slight changes in ambient oxygen content and make physiological adjustments to compensate for it. Both spinally blocked and swimming fish showed similar linear relationships between percentage reduction in ambient PO_2 and HR. The use of restrained, spinally blocked fish is a useful methodology and is advantageous in enabling the use of multiple instrumentation techniques that are impossible in free-swimming fish.

[10]The National Marine Fisheries Service provided financial support and space at their Kewalo Basin Research Facility for this research. Sponsor: Dr. G. Causey Whittow.

[11] University of Hawaii, Department of Physiology.

Qualitative Histochemical Evaluation of Glycosaminoglycans in Hamster Ovarian Folliculogenesis[12]

M. J. DAUGHEN[13]

This study investigated the role of glycosaminoglycans (GAG's) in folliculogenesis. Female hamsters ($n = 16$) from each day of the 4-day estrous cycle and during ovulation (day 4, 2000 hours to day 1, 1000 hours) were studied. Progesterone/estrogen ratios were evaluated by RIA. Histochemical composition of ovaries was examined using a PAS-alcian blue (pH 2.5) method. Serial sections (7 μm) of the ovary were compared in their affinity for either alcian blue (GAG's), PAS (complex carbohydrates), or both. Structures examined were (1) developing and atretic follicles (AF); (2) corpora lutea (CL); (3) corpora albicans (CA); and (4) the cumulus-oocyte complex (COC). Granulosa cells of all follicles and of the COC were PAS-positive. Follicular fluid of antral and preovulatory follicles reacted with both stains. In these follicles peripheral granulosa cells produced a definitive blue border, but follicular fluid was predominantly PAS-positive. Fluid from AF stained by alcian blue. The zona pellucida reacted positive to PAS during follicular development and atresia, but reacted with alcian blue as early as 2 hr postovulation. CL reacted only with PAS whereas CA reacted with both stains. Results indicate that ovulation induces permeability changes of the zona pellucida. Only antral follicles produce GAG's. The proportion and possibly the composition of GAG's produced by the granulosa cells may indicate follicular health.

Primary Culture of Crustacean Neurons in Defined Medium[14]

ROBERT A. GRAF[15]

Nervous systems of invertebrates have provided material especially useful for studies correlating morphology and function at the cellular level. However, studies using preparations of crustacean, molluscan, annelid, and insect neural tissue have shown that many interesting functional sites occur on the neurites and distal processes. These are inaccessible to many experimental manipulations because they are embedded in neuropil. To overcome this problem, neurons have been dissociated and placed in primary culture. When provided a suitable medium, usually supplemented with undefined growth factors, the neurons exhibit outgrowth. In the culture systems examined, neurons retain many of their distinctive in situ characteristics.

Primary cell culture systems have been developed for molluscan, annelid, and insect as well as vertebrate neurons, but I am unaware of any for crustacean neurons.

I report neurite outgrowth in defined medium from crustacean neurons isolated from a distinct group of peptidergic neurosecretory cells, called the X-organ, of crabs (*Cardisoma carnifex*) and lobsters (*Panulirus marginatus*). Cells from *Cardisoma* were confirmed as X-organ neurons from morphological, immunocytochemical, electrophysiological, and hormone-release evidence. The neurons have two predominant morpho-

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[13] University of Hawaii, Department of Animal Science and Department of Anatomy and Reproductive Biology.

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[15] University of Hawaii, Department of Zoology and Bekesy Neurobiological Laboratory.

logical growth patterns. Generally, neurons with small-diameter soma (<30 μm) sprout a branching neurite pattern whereas cells with larger-diameter soma (up to 70 μm) form large veils from the end of an axon stump, provided it adheres. Cells were held in culture for periods of up to a month.

Electron microscopy was performed on cultured cells (by S. Grau and T. Weatherby) and revealed neurosecretory granules throughout the soma, axon, and processes. Cultured cells showed positive immunoreactivity to antisera against three crustacean hormones, one hormone analog, and one hormone precursor sequence from the X-organ–sinus gland system (by S. Grau). Resting membrane potentials provided an indication of cell viability; values of -30 to -60 mV were typically recorded. Electrical activity included inward and outward currents recorded using whole-cell patch clamp techniques. High potassium stimulation of cells cultured from a single X-organ released red pigment–concentrating hormone by bioassay. The rate of secretion was 10 times that of the same culture unstimulated.

The culture and subsequent outgrowth of mature crustacean neurons provide a framework for examination of various physiological agents that may modulate and/or regulate the existing morphological and functional characteristics. This is particularly facilitated by the ability to make comparisons against outgrowth observed in defined medium. Since X-organ cells are peptide neurosecretory in nature, the cultures are promising for examination of questions concerning hormone biosynthesis, transport, and release.

Gap Junctional Communication (GJC) in C3H/10T1/2 Cells Is Induced by Retinoids[16]

MOHAMMAD ZAHID HOSSAIN[17]

Research was designed to test the hypothesis that direct intercellular communication through hydrophilic channels (gap junctional communication [GJC]) is involved in transporting growth regulatory molecules between cells in contact. This hypothesis is derived from observation that increasing GJC between confluent growth inhibited normal and transformed (tumorigenic) cells and blocked growth of the latter. Retinoids (vitamin A derivatives) have been shown to increase the growth control of normal 10T1/2 cells as well as to inhibit the process of neoplastic cell transformation. This study questioned whether this enhancement of growth control induced by the retinoids is associated with any change in GJC level. GJC was assayed by microinjecting lucifer yellow, a small-molecular-weight, water-soluble fluorescent dye, known to pass freely through gap junctions, into a single cell in a contacting-cell population and counting the number of neighboring cells that became fluorescent after 10 min. Retinol (10^{-6} M), retinoic acid (10^{-6} M), and the benzoidal derivative of retinoic acid, Ro 13-7410 (10^{-9} M), were highly effective in enhancing GJC in 10T1/2 cells. All three drugs increased GJC 7–10-fold more than the control (acetone treated) group. At these concentrations, the three drugs were equipotent in inhibiting the cell transformation caused by chemical carcinogens. Ro 13-7410 was clearly most potent in its effect on transformation and GJC; 2–3 days were required to exert its full GJC-inducing effect. This induced GJC was blocked by tetradecanoyl phorbol acetate (TPA), a potent tumor promoter, which was previously shown to antagonize the retinoid action on transformation. When added to a transformed cell

[16] Supported by the East-West Center and grant CA 39947 from the National Cancer Institute. Sponsor: Dr. John Bertram.

[17] University of Hawaii, Department of Biochemistry and Biophysics.

culture, Ro 13-7410 decreased the saturation density and altered cell morphology in a concentration-dependent manner, and this effect was accompanied by an increase in GJC.

When seeded over a confluent monolayer of 10T1/2 cells, transformed cell growth was not inhibited by Ro 13-7410, nor was heterologous GJC induced. In contrast to Ro 13-7410, elevated cellular cyclic adenosine monophosphate (cAMP) increased heterologous GJC but caused no induction of homologous GJC among 10T1/2 or transformed cells. This elevated heterologous GJC was associated with inhibition of transformed cell growth. Retinoids enhanced homologous GJC and exhibited increased growth control (i.e., decreased both saturation density and cell transformation) in pure culture, whereas cAMP increased heterologous GJC and inhibited transformed cell growth in coculture (mixed population of 10T1/2 and transformed cells). These data also indicate the probable existence of more than one gap junction with different regulatory control.

Environmental Cues Controlling Spawning in Two Species of Hawaiian Corals, *Montipora verrucosa* and *M. dilitata*[18]

CYNTHIA L. HUNTER[19]

Factors controlling reproductive and spawning periodicity in corals have been postulated but not empirically determined. Influences of lunar phase, seawater temperature, and day length on timing of gametogenesis and spawning were investigated in two congeneric Hawaiian corals. Five replicates from each of nine clones of *Montipora verrucosa* (Lamarck) and two clones of *M. dilitata* Studer were monitored under the following treatments: (1) constant artificial "full moon"; (2) shifted lunar phase (offset 14 days); (3) constant "new moon"; (4) flow-through seawater temperature lowered 0.5°C every 3 days and then maintained at 22.5°C for 1 month; (5) day length decreased by 10 min/day for 12 days and maintained on "short-days" for 1 month; and (6) controls (ambient summer seawater temperature, day length, and lunar phase).

Control colonies of *M. verrucosa* spawned (one to four spawnings per colony) between 2100 and 2130 hours on the nights of the new moon or the two nights following in June and July; *M. dilitata* colonies spawned on or within nine nights after full moon in July and August. Colonies of both species in the constant full moon treatment spawned synchronously with controls. In the shifted moon treatment, *M. verrucosa* and *M. dilitata* spawned on the same nights as controls for the first spawning cycles, but then sporadically thereafter (5–12 days off-cycle). Only two out of six *M. verrucosa* colonies in the constant new moon treatment spawned, both 1 week before new moon in August. *M. dilitata* colonies in this treatment retained synchronicity at the July full moon, but then spawned 8 days before controls in August. Decreased seawater temperature seemed to have precluded spawning in June for *M. verrucosa* and in July for *M. dilitata*, but *M. dilitata* colonies released large numbers of gametes at full moon in August. Only one *M. verrucosa* colony spawned in July, although mature gonads were noted within all colonies. In the short-day treatment, *M. verrucosa* colonies spawned on the same nights as controls, but 1.5 to 2 hr earlier. *M. dilitata* clones spawned in experimental and control chambers about 1.5 hr after moonrise. Temperature, lunar phase, and onset of darkness all act to control spawn-

[18] The Charles H. and Margaret B. Edmondson Fellowship, Community Scholarship Program, Alvin Y. Yoshinaga Fund, and the Hawaii Institute of Marine Biology are gratefully acknowledged for their generous support. Sponsor: Dr. Robert A. Kinzie.

[19] University of Hawaii, Department of Zoology and the Hawaii Institute of Marine Biology.

ing periodicity in corals. The physiological mechanisms by which these environmental cues are perceived and translated have not yet been determined.

Reproductive Patterns of the Chevron Butterflyfish, *Chaetodon trifascialis*: The Unfinished Puzzle[20]

DARBY IRONS[21]

The chevron butterflyfish, *Chaetodon trifascialis*, was studied to determine spawning patterns and fecundity estimates. Specimens were collected at Johnston Atoll. Gonads were examined using histological techniques and on the basis of gross morphological characteristics. The chevron butterflyfish is a gonochoristic, broadcast spawner with typical teleost gonads. Females appear to have asynchronous or possibly group-synchronous ovaries. Ovaries contained multiple egg size classes throughout the year, and the majority of eggs had a diameter greater than 0.4 mm. Most juveniles have sexually differentiated but immature gonads. Exceptions were individuals with primary oocytes and what appeared to be spermatogonia within the same gonad. Spawning peaks occur in the spring and fall. Spawning is postulated to occur at dusk. It is interesting that spawning has never been observed in several hundred hours of field observations. One possible reason for this could be that females may only be able to spawn during a narrow time window.

Regeneration of the Lateral Line System in *Ambystoma mexicanum*: A Time-lapse Videomicroscopy Study[22]

JAY E. JONES[23]

The posterior lateral line system of the aquatic salamander *Ambystoma mexicanum* consists of nerves and individual mechanoreceptive neuromasts. Neuromasts are discrete, rosette-shaped epithelia containing sensory hair cells and supporting cells that are remarkably similar in morphology, physiology, and embryonic history to their counterparts in the animal's ear. Amputation of part of the tail of *A. mexicanum* results in regeneration of the lost portion and replacement of approximately the same number of neuromasts as were removed. Previous work has indicated that an undifferentiated cellular mass, the regenerative placode, gives rise to the replacement neuromasts. Cells of the placode originate from the distalmost neuromast remaining after amputation. The object of this study was to specifically identify the type of cell that gives rise to the placode and to determine how the placode advances during regeneration. Differential Interference Contrast (DIC) microscopy, combined with time-lapse videorecording, was employed for direct observation of the distalmost neuromast remaining after partial tail amputation in axolotl larvae of a pigment-deficient strain. This technique demonstrated that supporting cells in the neuromast were mitotically active and

[20] This research was funded under U.S. Army contract number DACA83-84-C-0019. Sponsor: Dr. Ernst Reese.

[21] University of Hawaii, Department of Zoology.

[22] Supported by grants from NINCDS to Dr. J. T. Corwin. Sponsor: Dr. J. T. Corwin.

[23] University of Hawaii, Department of Physiology.

occasionally became motile, leaving the neuromast and moving out in the posterior direction appropriate for placode formation. Cell divisions were frequent in the placode itself and could account for its growth once seeded by cells that had migrated from the neuromast. Production of new cells must account for some part of the placode's extension, but placodal cells also became actively motile, as evidenced by membrane ruffling. Ruffling is usually a result of lamellipodia-mediated cell migration and is seen in cells at the leading edge of the advancing epithelial sheet during healing of wounds in mammalian skin. The ruffling seen in the placodal cells differed from that in wound healing in that all cells within a given area of the placode were involved, not just those of the leading edge. Macrophages were active at the regeneration site. In other systems these cells have been shown to secrete specific mitogens, inducers of mitotic activity, suggesting the possibility of such a role here.

Secondary Sexual Characters in *Lycosa howarthi*[24]

Bailey D. Kessing[25]

Ever since Darwin drew a sharp distinction between natural and sexual selection, biologists have struggled to understand the effects of sexual selection. Evolution of secondary sexual characters in males is thought to be the result of either Fisherian "runaway" selection, adaptive preferences by females, or male-male competition. Recent models of runaway selection indicate that sexual selection can lead to rapid evolution of secondary sexual characters and can be important in speciation events.

Wolf spiders (family Lycosidae) have been extensively studied for their striking secondary sexual characters and elaborate courtship behaviors in males. Representatives from this family (*Lycosa* sp.) are found in a variety of habitats on the main islands in the Hawaiian archipelago. A blind, cave-adapted species (*Lycosa howarthi*) is even found in the deep lava tubes on the island of Hawaii. *Lycosa howarthi* diverged from its surface ancestor (*Lycosa* sp.) after the formation of the island of Hawaii no more than 400,000 yr ago.

Using electron microscopy and audio-video taping, I have identified secondary sexual characters and courtship behaviors in the surface *Lycosa* sp. similar to those reported for related species studied in North America. The characters include hair tufts or "bottle-brush" structures on the first pair of legs, rasping surfaces for sound production at the tibio-tarsal joint on the pedipalps (a stridulating organ), and macrosetae at the tips of the pedipalps. In the cave-inhabiting *Lycosa howarthi*, males have lost most of the secondary sex characters found in their close surface relative. The only exception is that the stridulating organ is still present and unmodified at the tibio-tarsal joint of the pedipalps.

Courtship behaviors of the male surface *Lycosa* sp. involve stridulation, pedipalp drumming and scraping, bottle-brush waving, and throwing the first pair of legs against the substratum. Cave males have lost all the ancestral courtship behaviors. Instead of elaborate displays, cave males have evolved a novel courtship behavior, which consists of slowly approaching a cave female and, at irregular intervals, "bobbing" the abdomen three times. The stridulating organ on the pedipalps is unused and appears relictual.

Studies of sexual selection usually focus on the evolution and maintenance of extreme secondary sexual characters. Sister species in

[24] This research was funded in part by Sigma Xi (1985), the Cave Research Foundation (1987), and an NSF grant to Dr. Stephen R. Palumbi (1985–1988). Sponsor: Dr. Stephen R. Palumbi.

[25] University of Hawaii, Department of Zoology.

which sexually selected characters are completely lost or radically changed are rarely found. Studying the loss of ancestral characters and courtship behaviors and rapid replacement by other behaviors in *Lycosa howarthi* would offer the opportunity to examine aspects of sexual selection (e.g., skewed sex ratios and male competition) not as easily examined in other, more stable, systems. In the shift from surface to cave environment major changes in the balance of natural and sexual selection may have resulted in the rapid changes in secondary sexual characters and courtship behaviors.

A Day in the Life of Deepwater Copepods[26]

ANN KITALONG[27]

From 11 April to 13 May 1986, zooplankters were collected every 2 hr for 24 hr, on alternate days, from seawater pumped from 586 m to the surface at the National Energy Laboratory of Hawaii at Keahole, Hawaii. Composition and quantity of zooplankton was similar to that of zooplankton collected at other oceanic sites at similar depths. A diurnal increase for the mean settling volume (.004 ml/m^3) and mean abundances of zooplankters (including the vertically migrating copepods *Scottocalanus securifrons*, *Pleuromamma xiphias*, and *Aetidius armatus*, and fish [90% cyclothonids]) occurred from late morning to early afternoon (tested by two-way analysis of variance). A semidiurnal increase for the abundances of fish eggs occurred during late morning and midnight. Evidence for lunar periodicity was also found: during new moon, full moon, or both, there were increases in the mean settling volume and abundances of many taxa.

A higher proportion of *S. securifrons* females had material in their guts than *S. securifrons* males (Wilcoxon signed rank test). Gut contents of *S. securifrons* consisted of bacterial rods, unidentified grapelike clusters, organic "chicklets," and crustacean parts. A similar proportion of *Eucalanus crassus* females and males had material in their guts. The proportion of *E. crassus* males with full guts increased from late morning to early afternoon (two-way analysis of variance). Gut contents of *E. crassus* consisted of large unidentified "pillows" and diatom tests. Mean concentrations of nitrate and phosphate were correlated with mean abundances of *S. securifrons*, *P. xiphias*, and *Euchirella curticauda* (Pearson product moment correlation).

Sex ratios were skewed toward females for all copepod species examined except *Phyllopus* spp. More male than female *P. xiphias* and *A. armatus* were collected during new moon and just before full moon, respectively (two-way analysis of variance). A total of 97 attached spermatophores was observed (*S. securifrons*, 17; *E. curticauda*, 15; *Undeuchaeta* spp., 25; *Euchirella pulchra*, 16; *Heterorhabdus* spp., 8; *Candacia longimana*, 6; and all other copepods, 10). More attached spermatophores were observed during new moon. *S. securifrons* tended to show increased numbers of spermatophores every 5 days. Unidentified nauplii were more abundant during full moon, but were not correlated with any one copepod species. An increase in the number of *E. curticauda* males coincided with or was directly followed by an increased frequency of females with attached spermatophores, and subsequently followed by an increase in the mean abundance of copepodids 2 weeks later.

Predictable temporal periodicities in abundance, feeding, and reproduction exist for a deepwater zooplankton community below the thermocline. Precise sampling of deep oceanic water, from an exact location, has provided a unique opportunity to study deepwater zooplankton ecology.

[26] This research was funded by the Environmental Center of the University of Hawaii, Sigma Xi, and the International Women's Fishing Association. Sponsor: Dr. Julie Bailey-Brock.

[27] University of Hawaii, Department of Zoology.

Predation by *Chaetodon trifasciatus* and Growth and Distribution of *Pocillopora damicornis* at Coconut Island, Oahu, Hawaii

RANDALL K. KOSAKI[28]

The butterflyfish, *Chaetodon trifasciatus*, is a monogamous obligate corallivore that defends territories against neighboring conspecifics. At Coconut Island, adult pairs of *C. trifasciatus* hold territories along the windward reef slope but are not found on the adjacent reef flat. As the most abundant piscine corallivores present around Coconut Island, they may have considerable effect on their preferred prey species of coral, *Pocillopora damicornis*. A series of experiments was conducted to test the hypothesis that predation by *C. trifasciatus* may affect the distribution and abundance of its prey species.

In feeding preference experiments, adult individuals of *C. trifasciatus* fed on *P. damicornis* significantly more often than on other species of corals abundant on the reef flat and slope at Coconut Island. *P. damicornis* is abundant on the reef flat but is relatively rare on the reef slope. *C. trifasciatus* was found to have the opposite distributional pattern, spending a large majority of its time foraging on the reef slope. The question of interest is whether there is a causal relationship between the two patterns of distribution.

Individual colonies of *P. damicornis* were divided into four treatment groups: (1) caged reef slope; (2) uncaged reef slope; (3) caged reef flat; and (4) uncaged reef flat. There were 10 replicates of each treatment group. Weight increases after 21 days were measured to determine the extent to which exposure to predation on the reef slope limits growth. Significantly reduced growth rates were found among the uncaged reef slope treatments when compared to the other three treatments. The lack of statistically significant differences in growth rates between the caged reef flat and uncaged reef flat treatments is interpreted to indicate that caging did not affect growth rates and that the differences found between the uncaged reef slope treatment and the other three treatments are due to other factors.

These results suggest that although environmental conditions on the reef slope are suitable for active growth of *P. damicornis*, its distribution may be limited to the reef flat by the feeding preferences and foraging behavior of *C. trifasciatus*.

Evolution of Sexual Dichromatism in the Hawaiian Akepa, *Loxops coccineus coccineus*[29]

JAAN KAIMANU LEPSON[30]

The subspecies of akepa (*Loxops coccineus coccineus*) endemic to the island of Hawaii displays one of the most extreme cases of sexual dichromatism among Hawaiian birds as well as among birds in general. Adult males are bright orange, whereas females are dull gray-green. Reproductively mature subadult males display delayed plumage maturation and have a harlequin pattern of orange and gray-green during their first two potential breeding seasons. Long-term study was initiated in January 1987 to determine which process of sexual selection (male rivalry or

[28] University of Hawaii, Department of Zoology. Sponsor: Dr. Ernst S. Reese.

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[30] University of Hawaii, Department of Zoology.

female preference) is responsible for the evolution of exaggerated male color and brightness. Both processes are equivalent possibilities for the Hawaii akepa because Hawaiian honeycreepers evolved from cardueline finches, in which females choose males rather than resources, and because the akepa, unlike all cardueline finches, nests in cavities that may be attractive to females and defended by males.

This study is being conducted at the Hakalau Forest National Wildlife Refuge on the east slope of Mauna Kea, following a preliminary study in the Ka'u rainforest on Mauna Loa 75 km to the southwest. New techniques of mist-netting with aerial nets suspended 10–20 m above the ground have proven effective at catching canopy-dwelling akepa for body measurements and for banding with unique color combinations to allow individual identification necessary for accurate determination of behavior. No statistically significant morphological or behavioral differences were found between the two populations, and the populations have been combined for further analysis.

Several factors indicate that male competition may have influenced the evolution of exaggerated plumage coloration. Males have been seen to engage in group displays, with up to 12 individuals in a single tree sequentially flying out in a circle while singing. Males are also heavier and larger than females, with longer tarsi, wings, and tails. In addition, the Hawaii akepa is an obligate cavity nester throughout its range, and suitable nesting sites may be limiting and defendable. However, males do not appear to attract females to cavities. Males associated with females respond most strongly to playbacks of recorded songs, indicating that males may at least defend females.

Observational and experimental evidence suggests that female preference for bright adult males is possible. Although subadult males are sexually mature, all 10 known nests have involved adult-plumaged males, and subadult males are almost never seen paired with females. Preliminary results from model presentation experiments indicate that females pay more attention to adult male models, whereas males are equally attentive to both models. This sex-specific response suggests that females discriminate among males of different plumage. Such discrimination is necessary for female preference to occur.

Stimulation, Inhibition, and Induction of "Early" Sex Change in the Pomacanthid Angelfish *Centropyge potteri*

MARVIN LUTNESKY[31]

Potter's angelfish, *Centropyge potteri*, is a protogynous hermaphrodite occurring in discrete social groups (harems) of one male with one to eight females. Early sex change (ESC) is sex change in the presence of a male (i.e., the female does not need to change sex to remain reproductive). The purpose of this study is to (1) describe the socio-demographic patterns of *C. potteri* in high- and low-density populations; (2) introduce encounter rate threshold hypotheses for the proximate control of ESC; and (3) test if ESC can be induced in captivity when supplying the conditions predicted suitable by the encounter rate threshold hypotheses.

Socio-demographic patterns of *C. potteri* were measured in high- and low-density populations offshore of and inside Kaneohe Bay, Oahu, respectively. Peripheral movements of

[31] University of Hawaii, Department of Zoology. Sponsor: Dr. Ernst S. Reese.

males and females were observed during scuba dives. Color-tagged markers were dropped on the substrate to record home ranges of males. Females within the male's home range were counted to determine harem size.

Male home ranges were defended against conspecifics and should be considered territories. Females shared a territory with a male. Territories were significantly larger ($\bar{x}$ = 197.3 m^2, $n = 5$) in the low-density population than in the high-density population ($\bar{x} = 44.8$ m^2, $n = 6$). Harem sizes were significantly ($\bar{x} = 1.1$ females, $n = 8$) smaller in the low-density population than in the high-density population ($\bar{x} = 4.0$, $n = 9$).

Current hypotheses (sex ratio threshold, size ratio threshold, and inhibition hypotheses) of the proximate control of sex change in fishes suggest that females assess the social environment (e.g., sex ratio) as a cue to change sex. They do not, however, incorporate the influences of space. Introduced here are three encounter rate threshold hypotheses: (1) the absolute encounter rate threshold hypothesis, which suggests that ESC is triggered when a female is above a threshold level of stimulation by encounters with females, but below a threshold level of inhibition from encounters with the male; (2) the relative encounter rate threshold hypothesis, which suggests that ESC is triggered when stimulation by female encounter is a threshold level above inhibition from male encounter; and (3) the inhibition hypothesis (encounter rate interpretation), which suggests that ESC is triggered when a female is below a threshold level of inhibition from male encounter. These hypotheses incorporate the influences of both space and social group into a pattern of contact that is the cue for sex change. Only the first two hypotheses assume that contact with a female stimulates sex change, but all three hypotheses assume that contact with a male inhibits sex change. All three hypotheses predict that ESC should occur given a high female/male sex ratio and enough space for two minimal-size territories.

ESC was induced for the first time in captive, haremic hermaphrodites. Fifteen females and one male ($n = 3$) were placed in a large enclosure (34 m long × 3 m wide × 2 m high). After 30 days the largest or second largest female became male and divided the harem.

Life Cycle Length Plasticity and Its Role in Determining Distribution of Mitochondrial DNA Genotypes in Periodical Cicadas[32]

ANDREW MARTIN[33]

Periodical cicadas of the genus *Magicicada* have attracted the attention of evolutionary biologists because of their peculiar life history (e.g., long-lived, emergence periodicity, and large population sizes). From an evolutionary perspective, *Magicicada* is a model system for elucidating how temporal components of a species' life history influence the distribution of genetic variability within a species.

There are three distinct species of *Magicicada*, of which the most common is *M. septendecim*. There are two different life cycle lengths, of 13 and 17 yr. In addition, variation across the range of the species in emergence periodicity has resulted in the existence of separate year classes, called broods. Cicadas within a brood are highly synchronized so that at a given geographic locality cicadas emerge only once every 13 or 17 yr and reproduce. Because of the periodicity of emergences, cicadas within a brood are reproductively iso-

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[33] University of Hawaii, Department of Zoology.

lated from cicadas within any other brood, except in the rare cases when 13- and 17-yr brood emergences are synchronous and sympatric. This suggests that each brood is an incipient species. Allozyme and morphometric analysis has supported this contention.

This research focused on determining levels of variability within and extent of genetic divergence between two broods of *M. septendecim*—brood X (17-yr) and brood XIX (13-yr)—to assess the influence that temporal and spatial reproductive isolation have on genetic structure of the species. A total of 77 individuals from 11 populations of brood XIX and 24 individuals from 5 populations of brood X was analyzed using mtDNA restriction site analysis. Brood X seems to be monomorphic across the entire range of the brood (from Georgia to New York to Illinois). Within brood XIX two distinct (>2% divergence) mitochondrial genotypes were identified, one of which is identical to the brood X genotype. This pattern is repeated for two independent nuclear markers. The data suggest that 13-yr brood XIX cicadas in a large region of central North America are descendants of 17-yr cicadas that switched their life cycle to 13 yr. This provides the first strong genetic evidence that life cycle length is a plastic trait in cicadas, supporting the contentions of previous researchers for existence of plasticity in life cycle length in cicadas. The change in life cycle length and resultant anomalous distribution of genotypes imply that the cicadas became temporally isolated from the parent brood, joined an already existing brood, and thus brought together two independently evolving gene pools.

Effects of Larval Duration and Weight on Life Span and Reproduction in the Aeolid Nudibranch *Phestilla sibogae*[34]

STEPHEN E. MILLER[35]

Developmental and ecological events that influence larvae of benthic marine invertebrates have long been considered to play an important role in distribution and abundance of populations. However, the manner in which these larval events affect the expression of postlarval life history traits has only recently begun to be explored. The data presented here show that larval duration does not have a statistically significant influence on postlarval duration and reproduction.

Uncleaved egg masses from adults of the aeolid nudibranch *Phestilla sibogae* were collected and held in flowing seawater. Larvae were manually hatched just before natural hatching, which occurs about 6 days after first cleavage. Sibling groups of newly hatched larvae were maintained equally as fed or starved cultures. Weekly subsamples of both groups were induced to metamorphose onto the coral *Porites compressa*. The newly settled benthic juveniles were grown out as isolated pairs, feeding ad libitum on the coral throughout the remainder of their life spans. Durations of juvenile and adult periods were recorded along with daily weight of laid egg masses.

Starved larvae continually lost weight over a 21-day larval period, while fed larvae maintained their weight over a 28-day period. Results show that (1) duration of the juvenile and adult periods is unaffected by duration of the larval period; and (2) an increase in larval duration increases duration of the life span without affecting the reproductive contribution of the individual. Weight of egg masses

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[35] University of Hawaii, Department of Zoology.

produced during the first week of reproduction was the same for all adults regardless of larval duration. Therefore, the increase in duration of the life span was due to a hiatus in the developmental program that occurred during the larval period and was not due to changes in allocation of energy from reproduction to growth and maintenance in early adult life.

These findings indicate that the duration of periods of active development such as the juvenile period was relatively inflexible and has been set by strong selective pressures that have molded the genetic and developmental programs being expressed. Factors that influence life span, such as larval duration, do not influence the adult period and so have no influence on reproductive output and fitness. Individuals recruiting to populations after a minimum larval period will gain no reproductive advantage over individuals that recruit after an extended larval period because the timing of larval and postlarval life history events are developmentally uncoupled.

Avian Population Estimates in Relation to Annual Breeding Cycle[36]

MARIE MORIN[37]

Since 1968, censuses of the endangered Laysan Finch (*Telespyza cantans*) have been made on Laysan Island using a standard technique. Censuses are easy to conduct on this small, remote coral island with its low, open vegetation. Finches are counted on 120 randomly chosen transects within the vegetated area of the island. Each transect is 91.4 m long and 5 m wide. Observers use maps to locate transects and measure distances by pacing. Because of the small size of the transects and the open vegetation, counts are assumed to detect all birds present. Four to six counters participate in each census, and the same individuals are used whenever possible.

In 1986 and 1987 censuses were conducted during prebreeding, breeding, or postbreeding periods, determined by observations of the finch breeding cycle. Finches began breeding an entire month later in 1987 relative to 1986 (13 May versus 12 April). Onset of the breeding cycle is variable from year to year.

In 1986, finches were censused on 27 February, 28 May, and 23 July. In 1987, censuses were done on 15 April and 28 May. The 1986 population estimate showed a decline between the prebreeding (February) and the breeding (May) censuses, and then an increase in the postbreeding (July) census. A similar decline, although smaller, was seen from the prebreeding (April) to the breeding (May) census in 1987. A postbreeding census was not made in 1987. Confidence intervals were similar for all of the census estimates.

The apparent decline in the population estimates made during the breeding period may be due to one or more interacting breeding behaviors (e.g., birds may be more wary during the breeding season, and females may be undercounted because they are laying eggs and incubating and hence are hidden from view [male parents do not incubate]). If this is true, then the assumption that the breeding season transect counts are total counts may be false, and a correction factor may be appropriate to compensate for the lower population estimates made during the height of the breeding season. Whenever possible the stage of the breeding cycle should be documented during these censuses, because population estimates may vary in conjunction with breeding cycle stage.

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[37]University of Hawaii, Department of Zoology.

Effects of Exogenous Androgens on Growth, Biochemical Composition, and Reproduction of the Coot Clam, *Mulinia lateralis*[38]

SHAUN M. MOSS[39]

Anabolic androgens are known to increase body weight, affect lipid and carbohydrate metabolism, and cause masculinization and/or sterilization when administered to cultured fish and livestock, but little is known about their effects on bivalves. The objective of this study was to determine the effects of testosterone and methyltestosterone on growth, biochemical composition, and reproduction of the coot clam, *Mulinia lateralis* (Say), in order to evaluate potential of exogenous hormones in bivalve aquaculture.

Six-week-old *M. lateralis* were fed particulate testosterone at a weekly weight-adjusted dose of 0.01% individual live weight or particulate methyltestosterone at 0.001%, 0.01%, and 1.0% individual live weight every other day for 6 weeks. Control clams received no hormones. The 62 clams used in each treatment were maintained in seawater at 21.5°C and fed a mixed diet of *Thalassiosira pseudonana*, *Isochrysis galbana*, and *Chaetoceros gracilis*. Methyltestosterone at the 0.01% level increased clam weight compared to that of controls fed algae alone. Methyltestosterone at the 0.001% level increased clam weight as well as protein and carbohydrate concentrations. All androgen treatments increased shell height, width, and length. Methyltestosterone at the 1.0% level caused clams to spawn 10 days earlier than control clams and increased spawning frequency. Similar effects on clam reproduction were observed with methyltestosterone at 1.0% individual live weight when clams were cultured at 25.5°C and fed only *I. galbana*. Under these conditions, 1.0% methyltestosterone administration resulted in a male/female ratio of 1.6 : 1.0 in spawned clams and caused clams to spawn more frequently than controls, which had a male/female ratio of 0.83 : 1.0. These results indicate that low doses of particulate methyltestosterone (0.001% and 0.01% individual live weight) can accelerate growth rates when fed to juvenile *M. lateralis*, while high doses (1.0% individual live weight) can increase spawning frequency and may cause sex reversal.

Androgens could be used in culture of commercially important bivalve species as growth promoters and in broodstock management. Concern about hormonal residues in treated bivalves being transferred to consumers, as well as the economic feasibility of androgen use on a commerical scale, must be addressed.

Why Do Corals Fight? Effect of Interspecific Aggression on Growth Rate in Corals

SANDRA L. ROMANO[40]

Interspecific aggression is a strategy that has evolved among corals living in an environment that is often space limited. Mechanisms used by corals in interspecific interactions and occurrence of interspecific aggression in different coral assemblages have been well documented. Little attention has been given to the evolution of these interactions among corals. To understand how such complex interactions have evolved among corals, it is necessary to

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[39] University of Hawaii, Department of Zoology.

[40] University of Hawaii, Department of Zoology. Sponsor: Dr. Stephen Palumbi.

understand the effect of these interactions on their relative fitness.

Controlled interaction experiments designed to assess effects of aggression on one component of coral fitness were carried out with colonies of *Cyphastrea ocellina* and *Pocillopora damicornis*, two common reef flat corals in Hawaii. When these two corals are in contact, *C. ocellina* will always damage *P. damicornis*. Pairs of corals were placed in soft-tissue contact and situated on the reef flat of Coconut Island at the Hawaii Institute of Marine Biology. All colonies were alizarin-stained. This marker was used as a baseline for measuring linear growth after 11 months. Increase in skeletal weight was determined by measuring the buoyant weight of the corals at the beginning of the experiment and after 3, 6, and 11 months. Results were analyzed by analysis of variance.

There was no significant difference in growth rate of *P. damicornis* colonies growing next to living or dead *C. ocellina*. There were 25 pairs in these and all treatments. Colonies of *C. ocellina* growing alone had a significantly higher growth rate than that of *C. ocellina* in all other treatments. Growth rate of control colonies of *C. ocellina* in contact with nonliving *P. damicornis* was significantly higher than that of colonies growing in contact with living *P. damicornis* as measured by buoyant weight. Linear growth of colonies of *C. ocellina* growing in contact with nonliving and living branches of *P. damicornis* was not significantly different.

Although *C. ocellina* is never damaged in an interaction with *P. damicornis*, its growth rate is lower than that of a colony growing alone. *P. damicornis* is always damaged in an interaction with *C. ocellina*, but damage does not result in reduced growth rate. This indicates that (1) aggression decreases the fitness of the winners; and (2) the losers of an interaction may not suffer greatly. Other components of fitness must be considered to determine the overall effect of interspecific aggression on the fitness of corals. Future interaction experiments will be used to determine the effect of aggression on reproductive output and on energetic state of corals in order to completely evaluate the relative costs and benefits of interspecific aggression among corals.

Growth Estimates of Commercially Exploited Antarctic Fish

DAVID J. SHAFER[41]

Increased exploitation and subsequent declining population sizes of commercially important antarctic fish obligates the urgent need for descriptive population information to qualify enlightened management and conservation decisions. Fundamental to the elucidation of accurate population dynamics is the determination of individual age. Age estimations can describe basic life history events, including age-related mortality and fecundity, recruitment success, age at maturity, population structure, changes in population growth, and other information critical to understanding population structure. Conventional age resolution efforts, utilizing various hardparts for analysis, have relied upon interpretation of rhythmic depositional reference marks, which are moderated by seasonal environmental fluctuations. These attempts have encountered great difficulty in age assessment of antarctic fish due to the inherent lack of distinct periodicity in antarctic hydrographic conditions. With the advancement of otolith daily increment methodology, it is now feasible to delineate relevant life history information encoded in calcium carbonate otolith concretions. *Champsocephalus gunnari* and *Notothenia rossii* were used as models to study both external and internal otolith morphology. Rhythmic patterns, judged to be daily in occurence, were discovered in the otolith microstructure, enabling estimation of chronological age. Multivariate mathematical models, relating

[41] University of Hawaii, Department of Zoology. Sponsor: Dr. Richard Radtke.

age to otolith morphometrics and fish size, were used to calculate age from body and otolith measurement data. It was determined that *C. gunnari* grew 55 cm in 17 yr and *N. rossii* grew 55 cm in 7 yr. These data indicate that antarctic fish are slow growing, and consequently their populations are greatly susceptible to overfishing. Further application and refinement of external and internal otolith methodology will provide valuable population information describing the population attributes and dynamics of antarctic fish.

Fragmentation: A Mechanism for Stimulation of Genet Growth Rates in an Encrusting Colonial Invertebrate[42]

DOUGLAS S. STONER[43]

This study tests the hypothesis that fragmentation in encrusting colonial marine invertebrates is a mechanism for stimulating genet growth rates. The hypothesis predicts that a clone of small colonies produced by fragmentation will grow faster than a single intact colony of equal biomass, because small colonies proportionately have a greater amount of periphery relative to surface area over which they can expand. If the hypothesis is true, then relative growth rates should decline with increasing colony size. This expectation was tested in a colonial ascidian, *Diplosoma similis*, by comparing the growth rates over a 2-week period of 41 sets of genetically identical colonies that ranged in size from 0.01 to 3.5 cm^2. Each set was genetically distinct from the rest and was produced by artificially fragmenting large naturally occurring colonies, a process that preliminary studies showed had no effect on growth rates. The relationship between growth (change in area) and initial size was modeled as a power function. The exponent of the power function, representing the rate of change with colony size, was empirically determined to be 0.85 ± 0.02, indicating that relative growth rates decline with increasing colony size as predicted.

Mating Patterns in the Common Myna, *Acridotheres tristis*[44]

TERESA M. TELECKY[45]

Twenty pairs of the Common Myna, *Acridotheres tristis*, were studied for three breeding seasons to document and analyze their mating patterns. Previous studies indicated that mynas are strictly monogamous within a clutch. Most pairs remain together for more than one breeding season and commonly produce two or three clutches per season. Such long-term association may be advantageous to individuals, or they may be constrained to remain together by lack of other breeding opportunities.

Observations of 32 breeding attempts indicate that pair-bonds persist through the nonreproductive season; re-pairing occurred only within breeding seasons and relatively quickly (9–14 days). Twenty-four (75%)

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[43] University of Hawaii, Department of Zoology.

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[45] University of Hawaii, Department of Zoology.

breeding attempts were produced by individuals that reunited with their previous mate and eight (25%) were produced by individuals that re-paired with a new mate. Three types of re-pairing were observed: one member of the pair disappeared, one member of the pair died, or both members were observed alive but one had a new mate. Individual reproductive success in clutches immediately preceding and following re-pairing did not differ, indicating neither short-term advantage nor disadvantage to re-pairing. Mating patterns were unrelated to the reproductive success experienced by the pair in the previous clutch.

Newly formed pairs, pairs that had experienced at least one clutch together, and pairs that had experienced three or more clutches together had similar reproductive success as measured by clutch size, hatching success, and fledging success. This indicates absence of long-term advantages to these mating patterns. Other possible advantages to reuniting includes the retention of a nesting territory; however, there are many available nest sites and reunited pairs were not consistently associated with the same nesting territory.

There is no evidence that mynas are constrained to reunite. Many vacant territories exist, and new pairs do not have to fight to establish a nesting territory. The rapidity of re-pairing indicates the availability of unmated individuals. Additionally, the social environment promotes free association of mated and unmated birds in foraging flocks and sleeping roosts.

α-thalassemia Variants and Anomalous ξ-globin Genes in a Laotian Population in Hawaii[46]

ELIZABETH A. TITUS[47]

Restriction haplotype analysis was used to determine the molecular basis of α-thalassemia in a select Laotian population. α-thalassemia is a quantitative hemoglobinopathy, usually caused by large deletions of α-globin DNA. Characterization of α-globin gene deletions in 17 families (52 patients) was accomplished using Southern blot technique. Ambiguous haplotypes were analyzed repeatedly and interpreted in the context of findings in family members. Genotype frequencies were estimated for α-thalassemia variants in this population using a gene-counting procedure with corrections for duplicate genes among related individuals. In addition to α-thalassemia variants, anomalous ζ-globin haplotypes were found segregating in several Laotian families. Normal human ζ-globin genes encode α-like embryonic hemoglobin subunits in the α-globin multigene complex on chromosome 16. There are normally two ζ-globin genes in this cluster: 5′ ζ_2 and 3′ $\Psi\zeta_1$. In this study, six individuals in three families had triple ζ-globin genes. Another family revealed a novel quadruple ζ-globin arrangement confirmed by Southern blot analyses using double digestions hybridized to inter- and intragenic ζ-gene DNA probes. Repetitive DNA sequences in the ζ-globin region could facilitate misalignments and unequal crossing over events responsible for multiple and diminished gene arrangements and length mutations in the α-globin cluster. Characterization of the crossover regions in the triple and quadruple ζ-globin genes of the α-globin complex will aid in understanding the processes of gene expansion and deletion so often responsible for genetic disease.

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[47] University of Hawaii, Department of Genetics.

Optimal Colony Size in the Hawaiian Sergeant, *Abudefduf abdominalis* (Pisces: Pomacentridae)[48]

WILLIAM A. TYLER III[49]

Colonial nesting behavior is widespread in diverse groups of animals. Several hypotheses have been proposed to account for this behavior, including enhanced predator defense and more efficient foraging. There may, however, be costs associated with nesting in colonies. These arise from being in close proximity to conspecifics, such as competition for mates and food, or from providing a concentrated food source for predators. These considerations imply that there may be a colony size in which the difference between benefits and costs is maximized and reproductive success is optimal.

The colonial nesting behavior of a coral reef fish, *Abudefduf abdominalis* (Family Pomacentridae) was examined on two patch reefs in Kaneohe Bay, Oahu, Hawaii. Males prepare and defend demersal nest sites, court females, and then guard nests, containing up to six clutches of eggs, from egg predators. Males may be found nesting as solitary individuals or in colonies of from 2 to 30 individuals. Colonies of different sizes are present in the same contiguous habitat, allowing direct comparisons of various measures of costs and benefits between colony sizes.

Data collected from April 1985 to June 1987 indicate that average nest loss for one reef population is 36.8%. However, nest loss is a decreasing function of colony size, with solitary nests and small (2–7 nests) colonies having significantly greater rates of nest loss than intermediate (8–14 active nests) and large (15 or more nests) colonies. The number of clutches spawned in intermediate-sized colonies is significantly greater than that in either small or large colonies, as is the number of nests containing three or more clutches.

Time budgets of parental behaviors of nesting males in colonies of different sizes demonstrate that solitary males and those in small colonies have significantly more egg predators approaching the nest site and chase more egg predators than do males nesting in larger colonies. Males in small colonies also spend less time caring for their eggs than do males in larger colonies. These results indicate that colonies of intermediate size may be optimal for reproduction in this population.

Comparison of Impact of Two Disturbances on Coral Assemblages[50]

WILLIAM A. TYLER III AND RACHEL FITZHARDINGE[51]

We have investigated the impact of two disturbances on a coral assemblage dominated by *Montipora verrucosa* and *Porites compressa*. The first disturbance occurred when large quantities of drift algae were blown onto the leeward side of Coconut Island, Oahu, Hawaii, in November 1986. Algae remained on the reef for over 2 months, gradually decomposing. Corals were killed within a 40-m-wide stretch of reef. The second disturbance, a freshwater kill that occurred in January 1988, affected Coconut Island and other fringing and patch reefs in Kaneohe Bay.

In January 1987, we marked out randomly positioned transects, three within the area

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[51] University of Hawaii, Department of Zoology.

severely disturbed by the algae and three outside that area. Transects ran from the reef flat down the reef slope. Percentage cover of live and recently dead corals was measured within a 1-m^2 quadrat at 2-m intervals. Major and minor axes lengths, colony height, and maximum height of damage were measured on each colony intersecting the section of the transects in which most mortality occurred. Percentage of damage to each colony was estimated visually. In addition, we experimentally investigated how the algae impacted the corals by covering *M. verrucosa* and *P. compressa* colonies with drift algae for 1 week. After the freshwater kill, we resurveyed the quadrats that we had marked out and remeasured coral colonies on the three transects not impacted by the drift algae.

Mortality from the first disturbance was highest from the reef crest to 3 m down the slope, but the effects extended down to at least 9 m. In contrast, effects of the freshwater kill were most severe on the reef flat and declined towards the reef crest, with little mortality evident more than 1 m below the reef crest. Both disturbances impacted *Montipora* more severely than *Porites*. Differences in colony height, area, or morphology between the two species could not explain this differential mortality. In our simulation of the disturbance caused by the algae, after removal of the algae colonies of both coral species appeared bleached with areas lacking live polyps. When we examined the corals 6 weeks later, mortality of *Montipora* was approximately twice that of *Porites*.

Both disturbances resulted in a change in the relative abundance of *P. compressa* and *M. verrucosa*. Previous field observations and experiments and immunological studies indicate that *M. verrucosa* can outcompete *P. compressa*. The early survival of *M. verrucosa*, however, is so poor that recruitment rates of this species are lower than those of *P. compressa*. We propose that in the absence of disturbances such as the two we have investigated, *M. verrucosa* and not *P. compressa* might be the most abundant coral within Kaneohe Bay.

Preliminary Survey of Thyroid Hormones in Serum and Oocytes of Tilapia, *Oreochromis mossambicus*, During Oogenesis[52]

GREGORY M. WEBER[53]

Thyroid hormones have been shown to have many developmental actions in vertebrates. In addition, exogenous thyroid hormone treatment has been shown to enhance larval development and survival in many teleosts, including tilapia, *Oreochromis mossambicus*. Endogenous thyroid hormones of maternal origin have also been found in teleost eggs. This study attempts to identify patterns of thyroid hormone accumulation in the developing oocytes of tilapia and to compare these levels to serum levels of the same hormones.

Tilapia used in this study were maintained in outdoor holding tanks. Thyroxine (T_4) and triiodothyronine (T_3) levels in the serum and oocytes were measured by a radioimmunoassay utilizing miniature sephadex columns. Thyroid hormones were extracted from the oocytes before being assayed.

Preliminary data showed that although serum levels of T_4 exceeded those of T_3, the reverse was found to be true in the oocytes. Quantities of both hormones increased as the oocytes developed. It appears that the oocytes accumulate thyroid hormones over a large portion of the oogenic cycle in ratios that differ from those measured in the serum.

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[53] University of Hawaii, Department of Zoology and Hawaii Institute of Marine Biology.

Role of Regeneration in Respiration of the Marine Polychaete *Cirriformia* sp.

DuWayne Worthington[54]

Marine polychaetes have long been known for their ability to regenerate missing regions of their bodies, but only a few investigations have experimentally addressed the role regeneration plays in the ecology of marine polychaetes. The polychaete family Cirratulidae is mainly characterized by a mass of long, exposed gill filaments located on the anterior portion of the head. They can be extended above the surface of the various sediments they live in to obtain oxygen directly from the water column. Preliminary experiments showed that these soft respiratory tissues can be regenerated in *Cirriformia* sp. Experiments were then designed to investigate the role that regeneration plays in respiration.

Seven individuals of *Cirriformia* sp. were placed in a small culture dish with 3 cm of sand. The culture dish was put into a larger plastic container, with an airstone placed just outside of the culture dish. The airstone was turned on and off five times, with 30-min intervals between observations. Appearance or disappearance of gills, number of gills above the sand, and height of the gills were recorded. In a second experiment, the anterior gills were either completely removed, removed from just one side of the polychaete, or not removed at all (control). The polychaetes were individually placed in 50-ml flasks filled with sand, and then randomly placed in a cluster around two airstones in a larger plastic tub.

The appearance of gills at the surface of the sand could be controlled by turning the airstone on and off. Only 15–20 gills were seen above the sand surface before turning off the air supply. Three minutes after it had been turned off, however, 70–80 could be seen. Extended depletion of air resulted in an increase in the number of the gills seen, followed by an extension of the gills to their fullest height (2.5 cm). In each case, restoration of air to the system resulted in reduction of exposed gill number and height.

All individuals were able to completely regenerate gills that had been removed. When gills were completely removed, the polychaete placed only its anterior head portion on the sand surface. Gills along the body of the polychaete were not raised above the sand level. With half of the gill mass removed, a maximum of 6–7 gill filaments could be spotted above the sand surface, while unamputated polychaetes raised 12–15 gills above the sand surface.

The exposure of gills above the sand surface should make the polychaete more vulnerable to routine gill loss due to wave action, movements by other animals, and predation. The fact that gills are regenerated by *Cirriformia* sp. suggests that with regard to respiration, regeneration plays an integral role.

[54] University of Hawaii, Department of Zoology. Sponsor: Dr. E. Alison Kay.